Partial Characterization of Mitogenesis Inhibiting Factor in Malaria Serum

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OBJECTIVE : To further characterize the properties of the mitogenesis inhibiting substance(s) in serum from patients infected with malaria.

BACKGROUND : Recently Wells, et al. (1) demonstrated that sera from patients with falciparum or vivax malaria significantly suppressed the blastogenic response of normal lymphocytes simultaneously stimulated with selected plant mitogens. Significant suppression was seen in the cellular response of lymphocytes stimulated with phytohemagglutinin (PHA) and Concanavalin A (Con A) but not in cultures stimulated with Pokeweed mitogen (PWM). Later experiments using autologous or allogenic responder lymphocytes showed similar results (2) although some cultures stimulated with PWM did show various decrease of suppression.

We are continuing to investigate the inhibiting substance and the possible regulatory role it may have in the host's response to malaria infection. Preliminary studies are underway to determine if both acute and convalescent serum contain the inhibiting factor, if inhibiting serum must be added simultaneously with the mitogen for inhibition to be seen, and if the level of inhibition is altered when different concentrations of inhibiting sera are substituted in the culture media.

We will later begin physiochemical studies to determine the inhibiting substances' molecular weight, if it is dialyzable and/or temperature sensitive, and if its' action is directed toward both B cells and T cells.

METHODS : The mitogenesis inhibition assays are performed as previously described (1, 3) with modifications in the methodology for those experiments in which different dilutions of serum are added, or in experiments where inhibiting serum is added after mitogen is added to the specific culture wells.

Acute serum is obtained just prior to patients receiving chemotherapy, while convalescent serum is obtained 14 and 28 days later. The respective samples are frozen at -20°C until the complete set can be tested in a single assay against identical responder cells.

Additional physiochemical and target cell specificity studies will be performed using modifications of standard methods (3, 4, 5).

RESULTS : Table 1 shows the effect various dilutions of inhibiting sera have on the response of normal lymphocytes to mitogen. The highest SI is seen when

normal serum alone, or 15% normal serum plus 5% patients serum, is added to the culture media.

When the percentage of inhibiting serum is increased, a decrease in stimulation index (SI) is seen. These results, including the suppression of PWM induced mitogenesis, are consistent with the recent findings of Wells, et al. (2). The most important finding, however, is that the percentage of inhibiting serum can be reduced to 10%, thus conserving serum for additional studies. Additional experiments are underway to confirm these results.

Table 2 shows the effect of adding mitogenesis inhibiting sera to mitogen cultures at different times. Although only PHA was used to induce mitogenesis in this experiment, the results indicate that inhibiting serum must be added at the same time as the mitogen to bring about the highest level of suppression. Additional experiments will be necessary to see if inhibiting serum added at times prior to the addition of mitogen results in a higher level of suppression than in those culture receiving inhibiting serum and mitogen simultaneously.

Two inhibition experiments comparing convalescent serum with acute serum have been performed to date. The results are inconsistent and may tend to indicate that the presence of inhibiting substance in convalescent serum may be related to the chemotherapy the patient receives (6). This is a preliminary report.

REFERENCES

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Table 1 Effect Dilution of Inhibiting Sera was on Mitogenic Responses of Normal Lymphocytes

Mitogen	20% NS[1] + 0% P[2]	15% NS + 5% P	10% NS + 10% P	5% NS + 15% P	0% NS + 20% P
PHA	40.0[3]	43.32	21.6	22.18	31.8
CON A	141.92	132.11	68.70	58.10	101.50
PWM	128.70	127.82	58.91	37.97	56.87

[1] NS = Normal human serum [2] P = Patient (P79-006 PV) serum [3] NS = Stimulation index

Table 2. Effect of Adding Mitogenesis Inhibiting Sera at Different Time to Mitogen Cultures.

Mitogen	30% NS[1] (control)		10% NS + 20% P[2] (day 0)		10% NS + 20% P (day 3)		10% NS + 20% P (day 5)	
	CPM	SI[3]	CPM	SI	CPM	SI	CPM	SI
PHA	3105.7 / 338.25	9.2	7106.0 / 1502.25	4.7	3221.25 / 452.0	7.1	3810.5 / 371.25	10.3

[1] NS = Normal serum [2] P = Patient serum (73 Pf) [3] SI = Stimulation Index